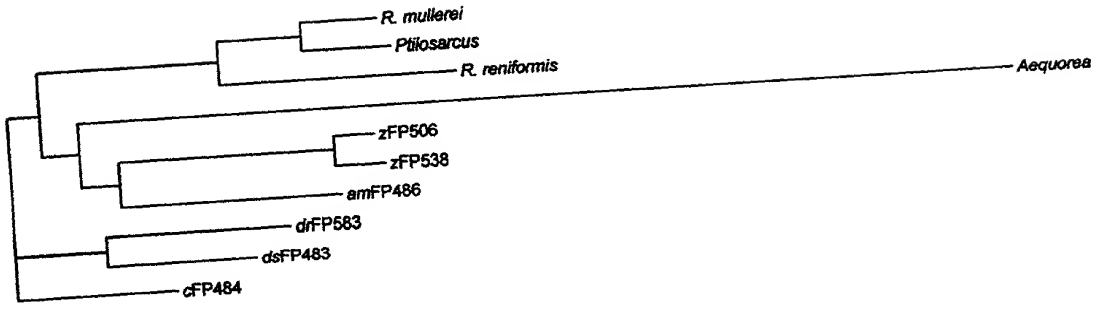
R. mullerei
Ptilosarcus
R. reniformis
Aequorea
zFP506
zFP538
amFP486
drFP583
dsFP483
cFP484

FIGURE 1

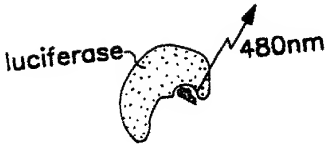

FIGURE 2A

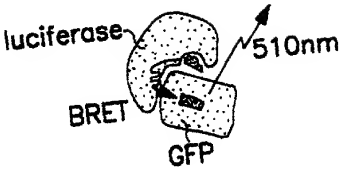

FIGURE 2C

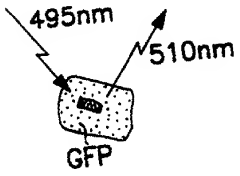

FIGURE 2B

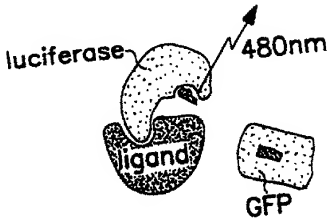

FIGURE 2D

FIGURE 2

BRET Sensor Architecture

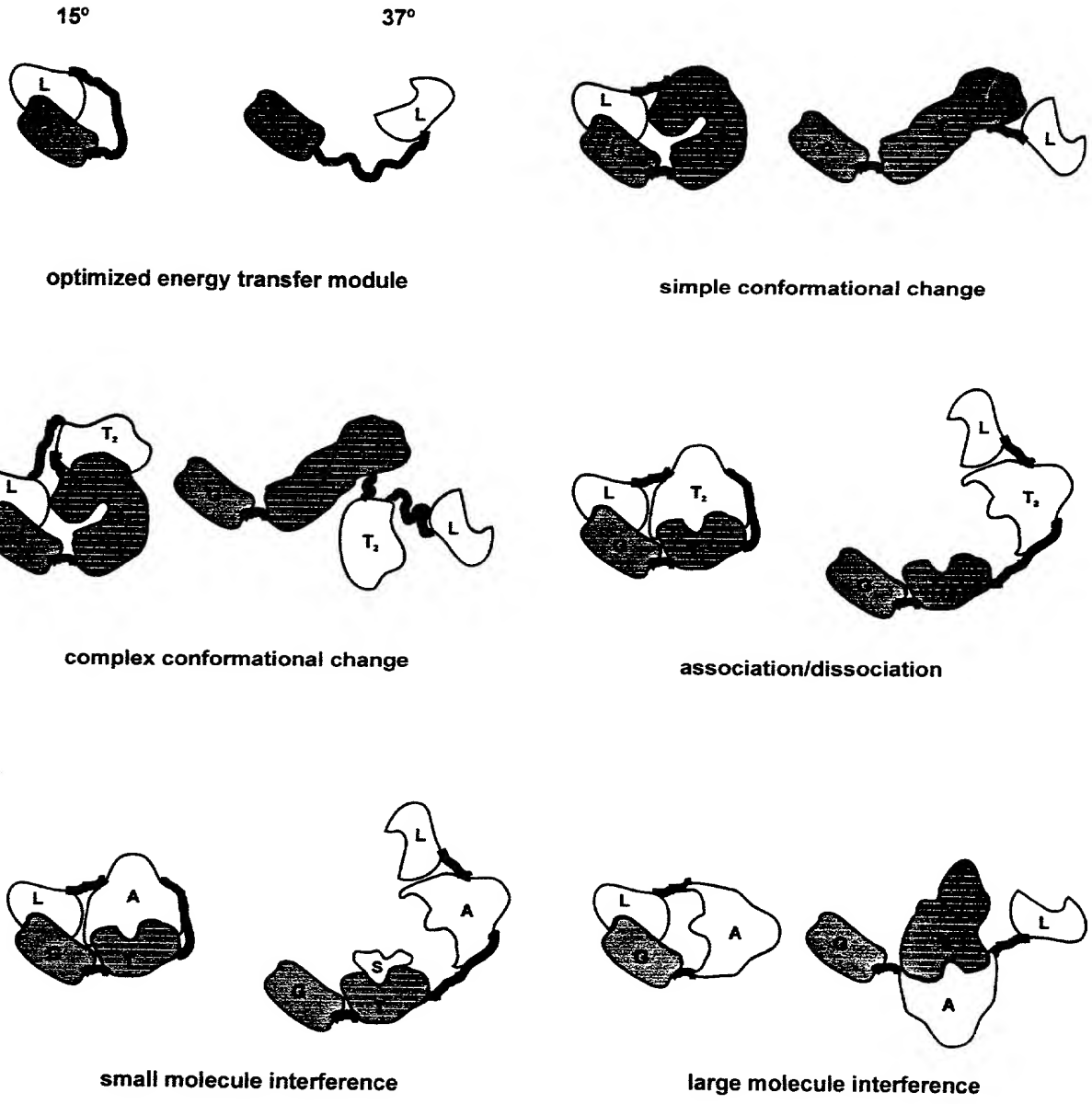

FIGURE 3

Utilization of advantageous GFP surfaces with substituted fluorophores

```
                60          *          80
RM-GFP    :  GAPLPFAFDIVSPAFQYGNRTFTKYPNDIS--  :  83
Pt-GFP    :  GGPLPFAFDIVSIAFQYGNRTFTKYPDDIA--  :  83
RR-GFP    :  GAPLPFAFDIVSVAFSYGNRAYTGYPEEIS--  :  80
cFP484    :  GAPLPFSYDILSNAFQYGNRALTKYPDDIA--  :  83
drFP583   :  GGPLPFAWDILSPQFQYGSKVYVKHPADIP--  :  80
asFP595   :  GGPLPFAFHILSTSCMYGSKTFIKYVSGIP--  :  77
dsFP483   :  GGPLPFGWHILCPQFQYGNKAFVHHPDNIH--  :  80
amFP486   :  GGPLAFSFDILSTVFKYGNRCFTAYPTSMP--  :  82
zFP506    :  GGPLPFAEDILSAAFNYGNRVFTEYPQDIV--  :  80
zFP538    :  GGPLPFSEDILSAGFKYGDRIFTEYPQDIV--  :  80
             ====================
```

FIGURE 4

```
                    *         20          *         40          *         60
R_reniform  : ---MDLAKLGLKEVMPTKINLEGLVGDHAFSMEGVGEGNILEGTQEVKISVTKGAPLPFAFDIVSV :  63
R_mullerei  : MSKQI.KNTC.Q..SYV.....I.NN.V.T....C.K...F.N.L.Q.R...............P :  66
Ptilosarcu  : MNRNV.KNT....I.SA.ASV..I.NN.V......F.K.V.F.N.LMQ.R....G..........I :  66
drFP583     : ---.RSS.NVI..F.RF.VRM..T.NG.E.FI..E..RPY..HNT..LK....G.....W..L.P :  63
                                    X

                  *         80          *        100          *        120          *
R_reniform  : AFSYGNRAYTGYPEEISDYFLQSFPEGFTYERNIRYQDGGTAIVKSDISLEDGKFIVNVDFKAKDL : 129
R_mullerei  : ..Q....TF.K..ND.....I......A..M....TL..E....LVEIR...N..IED..VYR.EY.GSNF : 132
Ptilosarcu  : ..Q....TF.K..DD.A....V......A..F.....L.FE..AIVDIR...........D..HYK.EYRGNGF : 132
drFP583     : Q.Q...SKV.VKH.AD.P...KKL.......KW...VMNFE...VVT.TQ.S..Q..C..YK.K.IGVNF : 129
                                                X X    O   X X X            X X XX

                 140          *         160          *         180          *     2
R_reniform  : RRMGPVMQQDIVGMQPSYESMYTNVTSVIGECIIAFKLQTGKHFTYHMRTVYKSKKPVETMPLYHF : 195
R_mullerei  : PDD.....KT.L..IE...F.A...M.NGVLV...V.LVY...NS..YYSC..K.LM...GV.KEF..S... : 198
Ptilosarcu  : PSN.....KA.L...E...F..VV.M.SGVLV...VDLVY...ES..NYYSC..K.F.R...GG.KEF..E... : 198
drFP583     : PSD.....KKTM..WEA..T..RL.PRDGVLK...IHK.L...KD..G.YLVEFKSI.MA...APVQL..G..YY : 195
               X                O O O O OX   X O O O        O O O     X X          O O

              00          *         220          *
R_reniform  : IQHRLVKTNVDTASGYVVQHETAIAAHSTIKKIEGSLP--- : 233
R_mullerei  : .......E..Y.EDGGF-..E...........QMTS.G..PL.....HEWV : 238
Ptilosarcu  : .H.....E..Y.EEG.F-..E...........QLT..G..PL.....HEWV : 238
drFP583     : VDSK..DI..SHNEDYTI..E..Y..RTEGR.HLFL---------- : 226
               O O                         O O O OO O
```

FIGURE 5

```
                    *        20         *        40         *        60         *        80
Aequorea    : ---MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTT---GKLPVPWPTLVTTFSYGVQCFSRYPDHMK :  79
R_mullerei  : MSKQILKNTCLQEVMSYKVNLEGIVNNHVFTMEGCGKGNILFGNQLVQIRVTK--GAPLPFAFDIVSPAFQYGNRTFTKYPNDI- :  82
Ptilosarcu  : MNRNVLKNTGLKEIMSAKASVEGIVNNHVFSMEGFGKGNVLFGNQLMQIRVTK--GGPLPFAFDIVSIAFQYGNRTFTKYPDDI- :  82
R_reniform  : ---MDLAKLGLKEVMPTKINLEGLVGDHAFSMEGVGEGNILEGTQEVKISVTK--GAPLPFAFDIVSVAFSYGNRAYTGYPEEI- :  79
drFP583     : ---MRSSKNVIKEFMRFKVRMEGTVNGHEFFIEGEGEGRPYEGHNTVKLKVTK--GGPLPFAWDILSPQFQYGSKVYVKHPADI- :  79
drFP593     : ---MSCSKNVIKEFMRFKVRMEGTVNGHEFEIKGEGEGRPYEGHCSVKLMVTK--GGPLPFAFDILSPQFQYGSKVYVKHPADI- :  79
dsFP483     : ---MSCSKSVIKEEMLIDLHLEGTFNGHYFFIKGKGKGQPNEGTNTVTLEVTK--GGPLPFGWHILCPQFQYGNKAFVHHPDNI- :  79
cFP484      : --KALTTMGVIKPDMKIKLKMEGNVNGHAFVIEGEGEGKPYDGTHTLNLEVKMAEGAPLPFSYDILSNAFQYGNRALTKYPDDI- :  82
asFP595     : ------MASFLKKTMPFKTTIEGTVNGHYFKCTGKGEGNPFEGTQEMKIEVIE--GGPLPFAFHILSTSCMYGSKTFIKYVSGI- :  76
amFP486     : ---MALSNKFIGDDMKMTYHMDGCVNGHYFTVKGEGNGKPYEGTQTSTFKVTMANGGPLAFSFDILSTVFKYGNRCFTAYPTSM- :  81
zFP538      : ---MAHSKHGLKEEMTMKYHMEGCVNGHKFVITGEGIGYPFKGKQ--TINLCVIEGGPLPFSEDILSAGFKYGDRIFTEYPQDI- :  79
zFP506      : ---MAQSKHGLTKEMTMKYRMEGCVDGHKFVITGEGIGYPFKGKQ--AINLCVVEGGPLPFAEDILSAAFNYGNRVFTEYPQDI- :  79

                 *      100         *       120         *       140         *       160         *
Aequorea    : RHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEG--DTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIK : 162
R_mullerei  : -SDYFIQSFPAGFMYERTLRYEDGGLVEIRSDINLIE--DKFVYRVEYKGSNFPDDGPVMQKTI-LGIEPSFEAMYM--NNGVLV : 161
Ptilosarcu  : -ADYFVQSFPAGFFYERNLRFEDGAIVDIRSDISLED--DKFHYKVEYRGNGFPSNGPVMQKAI-LGMEPSFEVVYM--NSGVLV : 161
R_reniform  : -SDYFLQSFPEGFTYERNIRYQDGGTAIVKSDISLED--GKFIVNVDFKAKDLRRMGPVMQQDI-VGMQPSYESMYT--NVTSVI : 158
drFP583     : -PDYKKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQD--GCFIYKVKFIGVNFPSDGPVMQKKT-MGWEASTERLYP--RDGVLK : 158
drFP593     : -PDYKKLSFPEGFKWERVMNFEDGGVVTVSQDSSLKD--GCFIYEVKFIGVNFPSDGPVMQRRT-RGWEASSERLYP--RDGVLK : 158
dsFP483     : -HDYLKLSFPEGYTWERSMHFEDGGLCCITNDISLTG--NCFYYDIKFTGLNFPPNGPVVQKKT-TGWEPSTERLYP--RDGVLI : 158
cFP484      : -ADYFKQSFPEGYSWERTMTFEDKGIVKVKSDISMEE--DSFIYEIRFDGMNFPPNGPVMQKKT-LKWEPSTEIMYV--RDGVLV : 161
asFP595     : -PDYFKQSFPEGFTWERTTTYEDGGFLTAHQDTSLDG--DCLVYKVKILGNNFPADGPVMQNKA-GRWEPATEIVYE--VDGVLR : 155
amFP486     : -PDYFKQAFPDGMSYERTFTYEDGGVATASWEISLKG--NCFEHKSTFHGVNFPADGPVMAKKT-TGWDPSFEKMTV--CDGILK : 160
zFP538      : -VDYFKNSCPAGYTWGRSFLFEDGAVCICNVDITVSVKENCIYHKSIFNGMNFPADGPVMKKMT-TNWEASCEKIMPVPKQGILK : 162
zFP506      : -VDYFKNSCPAGYTWDRSFLFEDGAVCICNADITVSVEENCMYHESKFYGVNFPADGPVMKKMT-DNWEPSCEKIIPVPKQGILK : 162

                  180         *       200         *       220         *       240         *
Aequorea    : VNFKIRHNIEDGSVQLADHYQQNTPIG-DGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDELYK---- : 238
R_mullerei  : GEVILVYKLNSGKYYSCHMKTLMKSKG--VVKEFPSYHFIQHRLEKTYVEDGGF--VEQHETAIAQMTSIGKPLGSLHEWV : 238
Ptilosarcu  : GEVDLVYKLESGNYYSCHMKTFYRSKG--GVKEFPEYHFIHHRLEKTYVEEGSF--VEQHETAIAQLTTIGKPLGSLHEWV : 238
R_reniform  : GECIIAFKLQTGKHFTYHMRTVYKSKK--PVETMPLYHFIQHRLVKTNVDTASG-YVVQHETAIAAHSTIKKIEGSLP--- : 233
drFP583     : GEIHKALKLKDGGHYLVEFKSIYMAKK--APVQLPGYYYVDSKLDITSHNEDYT-IVEQYERTEGRHHLFL---------- : 226
drFP593     : GDIHMALRLEGGGHYLVEFKSIYMVKK--PSVQLPGYYYVDSKLDMTSHNEDYT-VVEQYEKTQGRHHPFIKPLQ------ : 230
dsFP483     : GDIHHALTVEGGGHYACDIKTVYRAKK--AALKMPGYHYVDTKLVIWNNDKEFM-KVEEHEIAVARHHPFYEPKKDK---- : 232
cFP484      : GDISHSLLLEGGGHYRCDFKSIYKAK---KVVKLPDYHFVDHRIEILNHDKDYN-KVTLYENAVARYSLLPSQA------- : 231
asFP595     : GQSIMALKCPGGRHLTCHLHTTYRSKKPASALKMPGFHFEDHRIEIMEEVEKGK-CYKQYEAAVGRYCDAAPSKLGHN--- : 232
amFP486     : GDVTAFLMLQGGGNYRCQFHTSYKTK---KPVTMPPNHVVEHRIARTDLDKGGN-SVQLTEHAVAHITSVVPF-------- : 229
zFP538      : GDVSMYLLLKDGGRYRCQFDTVYKAK--SVPSKMPEWHFIQHKLLREDRSDAKNQKWQLTEHAIAFPSALA---------- : 231
zFP506      : GDVSMYLLLKDGGRLRCQFDTVYKAK--SVPRKMPDWHFIQHKLTREDRSDAKNQKWHLTEHAIASGSALP---------- : 231
```

D,E,H,K,R	N,Q,S,T	L,I,V,M,F,Y,W	A,G	C,P
polar charged	polar uncharged	non-polar hydrophobic	small	not grouped

dimerization surfaces: ☐ hydrophilic ☐ hydrophobic

FIGURE 6